II. REMARKS

This Preliminary Amendment is responsive to the Office Action mailed November 6, 2001 and the comments in the Advisory Action mailed June 7, 2002, in connection with the above-identified patent application. In addition, Applicants wish to thank the Examiner and her supervisor for their suggestions in an interview with Applicants' representative on June 4, 2002. Reconsideration of the application in view of the amendments, the new claims, and the following remarks is respectfully requested.

Upon entry of the amendment, claims 16-20 and 22-49 will be pending as shown in Exhibit B. A copy of the amended claims is attached as Exhibit A, called VERSION WITH MARKINGS TO SHOW CHANGES.

A. REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH

Claim 48 remains rejected under 35 U.S.C. §112, first paragraph. The Office Action states that while the specification is enabling for performing the method in a cell, and with gene expression of a reporter gene as the basis of the detectable signal, the specification allegedly does not reasonably provide enablement for performing the method in a cell with a non-gene expression based detectable signal or for performing the method *in vitro* with either type of detectable signal. Applicant respectfully traverses this rejection.

In vitro gene expression systems or "cell-free" systems have been known for over a decade. Accordingly, one skilled in that art would be able to perform the methods of the present invention in a cell-free system based upon teaching known to those of skill in the art at the time the present application was filed and based upon the teachings provided in the present specification.

In addition, non-gene based detectable signals were known in the art at the time of filing the present application. The specification teaches the quenching effect and fluorescence effect of GFP molecules in close proximity (pages 34-35). One of skill in the art would recognize from the present teaching that rather than utilizing a gene based reporter system, the association (or lack thereof) of two hybrid proteins (*i.e.*, a first protein with a first fluorescent molecule, and a second protein with a second fluorescent molecule) can be monitored due to fluorescent resonance energy transfer. In other words, the association of two fluorescent molecules changes upon the donor and acceptor wavelengths of fluorescence. Thus, when two fluorescent proteins are associated, there is a quenching of one fluorescent wavelength and an increase in another fluorescent wavelength. The changes in wavelengths can be monitored by standard optical methods.

While the illustrative examples in the specification as filed show gene-based reporter systems, the invention is not so limited. For example, on page 13, lines 3-28, the specification describes methods of detection for a "positive test" (see line 9) or screening and detecting "inhibition or enhancement of interaction of proteins or other molecules". The Office Action alleges that the present specification does not enable a non-gene based detectable signal. In addition, on page 35, lines 8-9, the specification describes incubation of cells containing two hybrid proteins in appropriate medium and monitoring the culture for a measurable activity. Such an activity is not limited to reporter gene expression, but rather includes growth of the cells in culture.

Applicant respectfully reminds the Examiner that a patent need not teach, and preferably omits, what is well known in the art. *In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991); *Spectra-Physics, Inc. v. Coherent, Inc.*, 827 F.2d 1524, 3 USPQ2d 1737 (Fed. Cir. 1987); *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987); and *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d 1452, 1463, 221 USPQ

481, 489 (Fed. Cir. 1984). All that is necessary is that one skilled in the art be able to practice the claimed invention, given the level of knowledge and skill in the art.

Further, as discussed in the prior filed Declaration under 1.132 by inventor Jay M. Short, in addition to detecting reporter gene expression, cell growth, or inhibition of cell growth, can also be utilized as a detectable signal for interaction of two molecules or interference with the interaction of two molecules in the method of the invention. For example, as shown in Figures 1 and 2 of the Declaration, when two regions of dihydrofolate reductase (DHFR) protein are allowed to interact via plasmids containing Fos and Jun genes and their interacting regions, (referred to as the "bait" and "target" in Figure 1) in a DHFR deficient host cell, in the absence of an inhibitor, cell growth is unaffected and the host cell survives. In contrast, in a DHFR deficient host cell, when the interaction between the two regions of DHFR are disrupted by a third molecule, for example from a mixed population library, the host cell growth is affected since there is no DHFR activity in the cell and the cell dies. This screening method allows one to measure the effect of a third molecule on the interaction of two other molecules in the absence of detection of expression of a gene as a reporter molecule.

Accordingly, Applicant respectfully requests withdrawal of the §112, first paragraph, rejection.

Claim 48 stands rejected under 35 U.S.C. §112, first paragraph as allegedly not enabling to one of skill in the art. While Applicant respectfully traverses this rejection, it is believed that the amendments to claim 48 which clarify the identity of the third molecule overcomes this rejection.

B. REJECTION UNDER 35 U.S.C. §112, SECOND PARAGRAPH

Claims 16-20 and 22-35 stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Applicant respectfully traverses this rejection.

Applicant has amended claim 16 and claim 22 to substitute claim language to provide antecedent basis as suggested by the Examiner. Accordingly, Applicant respectfully requests withdrawal of the §112, second paragraph, rejection.

C. REJECTION UNDER 35 U.S.C. §103

Claims 16-20, 22-32, and 36-45 stand rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Erickson *et al.* in view Stein *et al.* (1996 J. Bact. 178:591-599) and Horikoshi (1995 Curr. Op. in Biotech. 6:292-297). Claims 16-20, 22-32 and 36-45 stand rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Erickson *et al.* in view of Short *et al.* (WO 97/04077) and Horikoshi. Applicant respectfully traverses this rejection. Applicant respectfully traverses these rejections.

Applicant respectfully submits that the Office Action has failed to set forth a *prima facie* case of obviousness. In the absence of Applicant's disclosure, there must be found, at the time of filing, motivation or teaching to combine the cited references. In this case, there is no such motivation outside the disclosure of Applicant's invention. The alleged teaching is found, not in the references, but in the claims being rejected. It is error to reconstruct the claimed invention from the prior art by using the rejected claim as a ∀blueprint.≅ *Interconnect Planning Corp. v. Feil*, 227 USPQ 543, 548 (Fed. Cir. 1985).

The primary reference, Erickson *et al.* allegedly discloses a method for identifying a molecule which modulates the interaction between at least a first and second protein. Erickson *et al.* does not teach or suggest a molecule from a library generated from a

mixed population of organisms as recited in Applicant's claims 16 and 36, upon which the remaining claims depend. Further Erickson does not teach identification of a third molecule responsible for inhibiting interaction between a first and second molecule, for example, wherein that third molecule is also expressed from and encoded by nucleic acid from the same source as the first and second molecules. Erickson *et al.* fails to teach or suggest each and every element of Applicant's invention.

Stein *et al.* allegedly teaches creating libraries from uncultivated marine microorganisms. Stein *et al.* does not teach or suggest identifying proteins or molecules that modulate protein protein interactions. Horikoshi allegedly teaches enriching populations of extremophiles for prokaryotic organisms. Horikoshi does not teach or suggest identifying proteins or molecules that modulate protein-protein interactions. The combination of the foregoing references fails to remedy the failure of Erickson et al. to teach that all of the interacting molecules are obtained from the library generated from nucleic acid from a mixed population of organisms. Further, it would not have been obvious that one could succeed in making a representative gene library from a mixed population of organisms that would contain any particular molecule capable of modulating an interaction between two other molecules in the library since the nucleic acid molecules would be obtained from many organisms, both in sheer numbers and in numbers of species that would be represented in the library. It would not be obvious that obtaining a modulator of interacting proteins could be found from the enormous number of DNA molecules that would be cloned in generating the library. Obviousness requires more than just a motivation to try to identify molecules in the library, thus, Applicant submits that it would not have been obvious to actually find such molecules in the library.

Further , none of the references provide any motivation or suggestion, to combine the teachings of the references, and even if they did, the result would not be the claimed invention. Applicant respectfully submits that the present rejection is based upon hindsight reconstruction

of Applicant's invention based upon a number of references that do not teach or suggest the combination and come up short of the invention, even when combined. Accordingly, Applicant respectfully requests withdrawal of the §103(a) rejection.

Claims 16-20, 22-32 and 36-47 stand rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Erickson *et al.* in view of Stein *et al.* and Horikoshi, as applied above, and further in view of Patanjali *et al.* Applicant respectfully traverses this rejection.

Applicant respectfully submits that the Office Action has failed to set forth a *prima facie* case of obviousness. There is no suggestion, teaching, or motivation to arrive at Applicant's invention of identifying molecules in a library made from a mixed population of organisms that modulate interacting molecules. As discussed above, Erickson *et al.* fails to teach or suggest the claimed invention. Stein *et al.* allegedly teaches creating libraries from uncultivated marine microorganisms. Stein *et al.* does not teach or suggest identifying proteins or molecules that modulate protein-protein interactions. Horikoshi allegedly teaches enriching populations of extremophiles for prokaryotic organisms but does not teach or suggest identifying proteins or molecules that modulate protein-protein interactions. Patanjuli *et al.* is combined with the foregoing references to allegedly teach normalization of cDNA. The addition of Patanjuli *et al.* does not remedy the deficiencies of the primary or prior references and thus does not provide a *prima facie* case of obviousness. Patanjuli et al., does not teach "normalization" of species of genomic DNA as described in the present application. Applicant optionally normalizes DNA prior to generation of a genomic DNA library from a mixed population of organisms. For example, as described and claimed in Applicant's related US Patent 6,001,574, normalization entails obtaining a genomic DNA population from a mixed population sample, (e.g., environmental sample); at least one of the steps selected from the group consisting of (i) amplifying the copy number of the DNA population so isolated and (ii) recovering a fraction of the isolated genomic DNA having a desired characteristic; and

normalizing the representation of various DNAs within the genomic DNA population so as to form a normalized library of genomic DNA from the environmental sample. Patanjuli et al. Specifically, Patanjuli et al. does not teach or suggest normalization as described and claimed in the present invention.

The combination of the foregoing references fails to teach the introduction into a host cell, interacting molecules encoded by a mixed population library, and identification of a third molecule from that library, which directly or indirectly modulates the interaction between the first and second molecules. Applicant respectfully submits that the present rejection is based upon hindsight reconstruction of Applicant's invention based upon a number of references that do not teach or suggest the combination or provide motivation to make the combination.

Further, it would not have been obvious that one could succeed in making a representative gene library from a mixed population of organisms that would contain any particular molecule capable of modulating an interaction between two other molecules in the library since the nucleic acid molecules would be obtained from thousands and thousands of organisms, both in sheer numbers and in numbers of species that would be represented in the library, even if an enrichment step was performed. It would not be obvious that obtaining a modulator of interacting proteins could be found from the enormous number of DNA molecules that would be cloned in generating the library. Obviousness requires more than just a motivation to try to identify molecules in the library, thus, Applicant submits that it would not have been obvious to actually find such molecules in the library. Accordingly, Applicant respectfully requests withdrawal of the §103(a) rejection.

Claims 16-20, 22-33 and 36-45 stand rejected under 35 U.S.C. §103(a) as allegedly unpatentable over either Erickson *et al.* in view of Short et al. and Horikoshi and further in view of Mendelsohn *et al.* (Curr. Op. in Biotech. 1994 5:482-486). Applicant respectfully traverses this rejection.

Erickson *et al.*, combined with Short and Horikoshi, *do* not teach or suggest the claimed invention, as discussed above. Mendelsohn *et al.* allegedly teaches the use of GFP as a detectable gene in two hybrid methods but does not teach a molecule from a library made from a mixed population of organisms as recited in Applicant's claims 16 and 36. Thus, even if there were some suggestion to combine Mendelsohn *et al.* with the other cited references., which there is not, the combination of references does not teach or suggest the use of such a library as a source of DNA in the method of the claimed invention. Further, Applicant's inventive contribution is not based on which detectable marker was selected for detection or screening for modulators of protein-protein interaction. Applicant's invention resides in the unexpected finding that utilizing a mixed population DNA library would result in identification of modulators of interacting molecules, not in the reporter molecule utilized to detect such interactions. The failure of the previously cited references to render the invention obvious, certainly cannot be cured by the addition of a reference that teaches the use of GFP as a reporter gene. Accordingly, Applicant respectfully requests withdrawal of the §103(a) rejection.

In view of the amendment and the above remarks, it is submitted that the claims are in condition for allowance and a notice to that effect is respectfully requested. The Examiner is invited to contact Applicants' undersigned representative if there are any questions relating to this application.

Please charge any additional fees, or make any credits, to Deposit Account No. 05-1355.

Respectfully submitted,

Date: December 5, 2002

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Enclosures: Exhibit A – Version with Markings to Show Changes
Exhibit B – Claims as They Will Read Upon Entry of the Preliminary Amendment

EXHIBIT A

Version with Markings to Show Changes

16. (Twice Amended) A method for identifying a DNA sequence which encodes a molecule or molecules which directly or indirectly modulate the interaction between at least a first and second molecule, comprising:

introducing into a host cell containing interacting molecules which generate or repress a detectable signal or growth of the cell, genomic DNA or clones of a DNA library generated from nucleic acid obtained from a mixed population of organisms and measuring the interaction of a first molecule and a second molecule in the presence of a third molecule encoded by the library or the genomic DNA or produced as a result of expression of one or more products encoded by the library or the genomic DNA, wherein interaction of the first and the second molecules in the absence of the third molecule produces a detectable signal or growth of the cell;

comparing the signal or growth of the cell in the presence and absence of the genomic DNA or library, wherein a difference between the [response] signal or growth is indicative of the presence of a molecule that modulates interaction between the first and second molecules; and

identifying a clone or DNA sequence which encodes a molecule or molecules which directly or indirectly modulates the interaction between the first and second molecules.

22. (Amended) The method of claim 16, wherein the detectable signal is encoded by a gene present in [a] the host cell.

45. (Twice Amended) The method of claim 36, further comprising, prior to step (i):

obtaining the mixed population of organisms from an environmental sample [containing a mixed population of organisms]; and

enriching the sample for prokaryotic organisms, thereby creating an enriched environmental sample, wherein said sample is used to generate the library.

48. (Twice Amended) A method for [identifying] screening for the presence of a molecule that affects the interaction between a first and second molecule, comprising:

(i) contacting in a cell a first molecule with a second molecule wherein at least one of the first or second molecules is derived from a library made from a mixed population of organisms, wherein association of the first and second molecules in the presence of a third molecule results in the presence of a detectable response by changing expression of a detectable gene or detectable gene product; and

(ii) comparing the detectable response in the presence of the third molecule and the first and second molecules with the detectable response in the absence of the third molecule, wherein a difference in response is indicative of a first and second molecule that interact and a third molecule that affects the interaction between the first and second molecules, thereby [and] identifying the presence of a [third] molecule that affects the interaction of the first and second molecule.

49. (New) The method of claim 48, wherein a nucleic acid sequence encoding the third molecule is determined.

CLAIMS AS THEY WILL READ UPON ENTRY OF THE PRELIMINARY AMENDMENT

16. (Twice Amended) A method for identifying a DNA sequence which encodes a molecule or molecules which directly or indirectly modulate the interaction between at least a first and second molecule, comprising:

introducing into a host cell containing interacting molecules which generate or repress a detectable signal or growth of the cell, genomic DNA or clones of a DNA library generated from nucleic acid obtained from a mixed population of organisms and measuring the interaction of a first molecule and a second molecule in the presence of a third molecule encoded by the library or the genomic DNA or produced as a result of expression of one or more products encoded by the library or the genomic DNA, wherein interaction of the first and the second molecules in the absence of the third molecule produces a detectable signal or growth of the cell;

comparing the signal or growth of the cell in the presence and absence of the genomic DNA or library, wherein a difference between the signal or growth is indicative of the presence of a molecule that modulates interaction between the first and second molecules; and

identifying a clone or DNA sequence which encodes a molecule or molecules which directly or indirectly modulates the interaction between the first and second molecules.

17. The method of claim 16, wherein at least one of the interacting molecules contains a DNA-binding moiety and at least one of the interacting molecules contains a transcriptional activation or a transcriptional repressor moiety.

36. (Amended) A method for identifying a molecule that affects the interaction between a first and second molecule, comprising:

(i) contacting in a cell a first molecule with a second molecule in the presence of a third molecule encoded by a nucleic acid sequence from a library made from a mixed population of organisms or in the presence of a library or genomic DNA encoding the third molecule,

wherein association of the first and second molecules in the absence of the third molecule results in the absence or presence of a detectable response by changing expression of a detectable gene or detectable gene product; and

(ii) comparing the detectable response in the presence of the third molecule with the detectable response in the absence of the third molecule, wherein a difference in response is indicative of the presence of the third molecule that affects the interaction between a first and second molecule.

37. The method of claim 36, wherein the detectable response is the expression or repression of a detectable gene.

38. The method of claim 37, further comprising, prior to (i):

providing a prokaryotic host cell containing the detectable gene; and

providing a first gene expressed in the host cell, the first gene encoding the first molecule.

39. The method of claim 38, further comprising, prior to (i):

providing a second gene expressed in the host cell, the second gene encoding the second molecule.

27. The method of claim 16, wherein the library is derived from an environmental sample.

28. The method of claim 23, wherein the third gene is derived from an environmental library.

29. The method of claim 16 or 28, wherein the environmental library is derived from an environmental sample comprising uncultured microorganisms.

30. The method of claim 29, wherein uncultured microorganisms comprise a mixture of terrestrial microorganisms, a mixture of marine microorganisms, or a mixture of terrestrial and marine microorganisms.

31. The method of claim 29, wherein the uncultured microorganisms are extremophiles.

32. The method of claim 31, wherein the extremophiles are selected from the group consisting of thermophiles, hyperthermophiles, psychrophiles, and psychrotrophs.

33. The method of claim 16 or 23, wherein the library is created by obtaining an environmental sample, enriching the environmental sample for eukaryotic organisms and selecting against prokaryotic organisms, isolating nucleic acids from the enriched sample, fractionating the nucleic acids, and cloning the isolated nucleic acids into a vector.

34. The method of claim 33, wherein the nucleic acids are amplified prior to cloning into the vector.

35. The method of claim 33, wherein the vector is an expression vector

18. The method of claim 17, wherein the DNA-binding moiety and the transcriptional activation moiety are derived from a single transcriptional activator.

19. The method of claim 17, wherein the DNA-binding moiety and the transcriptional activation moiety are derived from different proteins.

20. The method of claim 16, wherein the detectable signal is produced from a gene encoding a protein selected from the group consisting of β-galactosidase, green fluorescent protein, luciferase, alkaline phosphatase and chloramphenicol acetyl transferase.

22. (Amended) The method of claim 16, wherein the detectable signal is encoded by a gene present in the host cell.

23. (Amended) The method of claim 22, wherein the host cell further comprises a first recombinant gene encoding the first molecule, a second recombinant gene encoding the second molecule, or a third recombinant gene encoding the third molecule.

24. (Amended) The method of claim 23, wherein the host cell contains both the first gene and the second gene and each gene is expressed.

25. (Amended) The method of claim 23, wherein the host cell contains the first, second and third genes and each gene is expressed.

26. The method of claim 25, wherein the host cell is cultured under conditions that allows for expression of the genes.

40. The method of claim 39, further comprising, prior to (i):

providing a third gene expressed in the host cell, the third gene encoding the third molecule.

41. The method of claim 40, further comprising, prior to (i):

introducing said first, second and third genes into the host cell; and

allowing expression of the genes.

42. (Amended) The method of claim 36, wherein the third molecule contains a DNA binding domain and a transcriptional activation domain.

43. The method of claim 36, wherein the interaction between the first and second molecules forms a transcriptional repressor.

44. The method of claim 36, wherein the third gene is derived from an environmental library.

45. (Amended) The method of claim 36, further comprising, prior to step (i):

obtaining an environmental sample containing a mixed population of organisms; and

enriching the sample for prokaryotic organisms, thereby creating an enriched environmental sample.

46. (Amended) The method of claim 45, further comprising producing a normalized library, comprising :

isolating nucleic acids from said enriched environmental sample;

fractionating the isolated nucleic acids;

and

amplifying any single-stranded nucleic acids present in the sample.

47. (Amended) The method of claim 46, further comprising generating an expression library, comprising:

inserting the amplified and isolated nucleic acids into an expression vector.

48. (Twice Amended) A method for screening for the presence of a molecule that affects the interaction between a first and second molecule, comprising:

(i) contacting in a cell a first molecule with a second molecule wherein at least one of the first or second molecules is derived from a library made from a mixed population of organisms, wherein association of the first and second molecules in the presence of a third molecule results in the presence of a detectable response by changing expression of a detectable gene or detectable gene product; and

(ii) comparing the detectable response in the presence of the third molecule and the first and second molecules with the detectable response in the absence of the third molecule, wherein a difference in response is indicative of a first and second molecule that interact and a third molecule that affects the interaction between the first and second molecules, thereby identifying the presence of a molecule that affects the interaction of the first and second molecule.

49. (New) The method of claim 48, wherein a nucleic acid sequence encoding the third molecule is determined.